STUDIES ON THE PRESERVATION OF GLANDS

I. The Preservation of Adrenal Glands

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A CENTRAL factory in our country for the large-scale production of gland products can be thought of only when we solve the problem of storage and transport of the glands. It is a well-known fact that the gland tissue suffers autolysis with simultaneous destruction of hormonal activity unless proper precautions are taken to preserve these glands. Three methods suggest themselves for the proper preservation of glands: (1) The use of low temperatures, which will arrest enzyme activity—an entirely physical method, (2) use of chemical preservatives which inhibit bacterial action and (3) a combination of both.

The present investigation concerns itself with the changes in the adrenaline and vitamin C contents of the adrenal glands stored under various conditions. The methods employed for the estimation of adrenaline and vitamin C have already been described elsewhere (Dey, Krishnan and Srinivasan[1]). In all cases, unless otherwise stated, the glands after collection from the slaughtered animals, were transported to the laboratory packed in melting ice. The glands were kept for varying periods at different temperatures: (*i*) room temperature (30°-32° C.), (*ii*) Frigidaire temperature (3°-5° C.), (*iii*) in the frozen condition, the freezing being effected either by placing in the freezing chamber of the Frigidaire (a temp. of — 7° C.) or by packing in solid carbon dioxide (— 30° C.). In the first two cases a few drops of toluene were added as preservative. In a separate series of experiments (No. V in Tables I and II), the estimations were carried out on glands without the addition of toluene. Investigations were also carried out on the changes in the adrenaline content of glands which were stored in alcohol at room temperature for a period of one week. The values given in the following tables represent the mean of a series of estimations.

It has already been indicated (*loc. cit.*) that greater reliance has to be placed on the persulphate colorimetric method and the indophenol titration method for the accurate estimation of adrenaline and ascorbic acid respectively. As is obvious from Tables I and II,

TABLE I
Cattle glands (whole)

Method of storage	Period of storage	Adrenaline: Weight (mg.) per gram of gland		Vitamin C: Weight (mg.) per gram of gland	
		Folin	Persulphate	Indicator	Iodine
(i) Fresh glands transported to the laboratory in melting ice	2–3 hours after death of animal	3·05	1·85	0·91	1·09
(ii) Freezing with " dry ice "	2 days	3·05	1·73	0·89	1·15
(iii) Freezing in the Frigidaire	24 hours	2·74	1·72	0·80	1·09
	3 days	2·56	1·73	0·79	1·11
	7 days	2·63	1·58	0·65	0·99
	14 days	2·36	1·52	0·66	0·94
	1 month	2·55	1·59	0·77	1·11
(iv) Keeping at Frigidaire temperature with a few drops of toluene	24 hours	2·69	1·73	0·93	1·26
	2 days	2·68	1·68	0·69	0·98
	3 days	2·66	1·64	0·72	0·96
	4 days Putrefaction sets in	2·70	1·30	0·21	0·37
(v) Keeping at Frigidaire temperature without toluene	24 hours	3·13	1·75	0·98	1·32
	2 days Putrefaction sets in	2·44	1·59	0·85	1·18
(vi) Keeping at room temperature with a few drops of toluene	16 hours Putrefaction sets in	3·16	1·66	0·70	0·94
(vii) Preservation in alcohol	8 days	2·03	1·65	0·08	0·12

TABLE II

Sheep glands (whole)

Method of storage	Period of storage	Adrenaline		Vitamin C	
		Weight (mg) per gram of gland		Weight (mg.) per gram of gland	
		Folin	Persulphate	Indicator	Iodine
(i) Fresh glands transported to the laboratory in melting ice	2-3 hours after death of animal	2·54	1·57	1·30	1·73
(ii) Freezing with " dry ice " ..	2 days	1·81	1·07	1·09	1·49
(iii) Freezing in the Frigidaire ..	24 hours	1·79	1·06	0·97	1·48
	3 days	1·92	1·06	0·98	1·40
	7 days	1·88	0·90	0·96	1·41
	14 days	1·87	0·88	0·92	1·32
	1 month	2·02	0·69	0·94	1·26
(iv) Keeping at Frigidaire temperature with a few drops of toluene	24 hours	2·40	1·18	1·03	1·45
	2 days	2·01	1·01	0·82	1·10
	3 days Putrefaction sets in	1·96	0·78	0·62	1·03
(v) Keeping at Frigidaire temperature without toluene	24 hours	1·82	1·03	1·19	1·60
	2 days Putrefaction sets in	1·64	0·65	0·95	1·28
(vi) Keeping at room temperature with a few drops of toluene	16 hours Putrefaction sets in	1·61	0·90	0·82	1·17
(vii) Preservation in alcohol	7 days	1·37	1·44	0·016	0·027

sheep glands, as a rule, suffer greater decomposition in adrenaline and vitamin C than cattle glands, stored under identical conditions, due probably to the softer texture of the former. Glands in the frozen condition can be stored without undergoing deterioration for several weeks. At the end of one month, the loss of adrenaline (as estimated by the specific persulphate oxidation reaction) is only about 15 per cent. in the case of cattle glands. In the case of sheep glands, however, the decomposition of adrenaline under these conditions is extensive (more than 50 per cent.). The analyses of adrenal glands, both cattle and sheep, frozen for 2 to 3 days either with CO_2 snow or by placing in the freezing chamber, show that the loss of adrenaline is not very appreciable. At the frigidaire temperature of 3 to 5° C., with toluene as preservative, the glands keep well for 2 to 3 days, the loss in adrenaline being only about 15 per cent., after which time putrefaction sets in. If no toluene is added the glands putrefy some 24 hours earlier. Kept at laboratory temperature, with a few drops of toluene, the glands develop smell in 12 to 18 hours' time, although adrenaline, as estimated by the persulphate method, does not suffer any serious loss. In all the above cases vitamin C also showed progressive decomposition although no definite correlation is apparent between the relative destruction of the adrenaline and the vitamin. Preservation of glands in alcohol is very effective; at the end of one week the loss of adrenaline in the case of cattle glands is 11 per cent. and in the case of sheep glands 8 per cent. Vitamin C, however, suffers extensive destruction under these conditions.

Much stress is often laid on the post-mortem diffusion of adrenaline from the medulla into the cortex. The glands which were frozen for one month, were then dissected carefully, and the medulla and cortex assayed separately for the adrenaline and vitamin C contents. The figures in Tables III and IV show that the medulla contains 63 and 71 per cent. of the total adrenaline in the case of the cattle and sheep glands respectively, as compared with 82 per cent. in the case of the fresh glands, showing thereby that a certain amount of diffusion has taken place.

In still another series of investigations (Table V) the glands, soon after collection from the slaughtered animals, were chilled by dropping into 'dry ice' and transported in this condition from the slaughter house to the laboratory, where they were immediately worked up. The values so obtained (*vide* Table V) show

TABLE III
Cattle glands (dissected)

Method of preservation	Adrenaline				Vitamin C			
	Weight (mg.) per gram of tissue				Weight (mg.) per gram of tissue			
	Medulla		Cortex		Medulla		Cortex	
	Folin	Persulphate	Folin	Persulphate	Indicator	Iodine	Indicator	Iodine
(i) Fresh glands ..	6·57	4·8	1·27	0·37	0·93	1·33	1·06	1·38
(ii) After freezing in the Frigidaire for one month	5·72	3·80	1·86	0·86	0·83	1·17	0·43	0·80

TABLE IV
Sheep glands (dissected)

Method of preservation	Adrenaline				Vitamin C			
	Weight (mg.) per gram of tissue				Weight (mg.) per gram of tissue			
	Medulla		Cortex		Medulla		Cortex	
	Folin	Persulphate	Folin	Persulphate	Indicator	Iodine	Indicator	Iodine
(i) Fresh glands ..	6·50	5·52	1·17	0·31	0·99	1·46	1·45	1·87
(ii) After freezing in the Frigidaire for one month	2·80	2·21	1·45	0·20	0·54	0·88	0·45	0·68

TABLE V
Instantaneous chilling of the glands with solid carbon dioxide

Animal	Adrenaline		Vitamin C	
	Weight (mg.) per gram of gland		Weight (mg.) per gram of gland	
	Folin	Persulphate	Indicator	Iodine
(i) Cattle	3·71	2·24	1·24	1·65
(ii) Sheep	2·52	1·60	1·36	1·71

a definite increase for both adrenaline and vitamin C, over those obtained for glands transported to the laboratory in melting ice.

A detailed discussion and interpretation of the results obtained will be published elsewhere. It is apparent, however, that for obtaining the maximum yield of adrenaline, the ideal procedure would be to chill the glands soon after collection from the slaughtered animals and to work them up within twenty-four hours. If the glands could be kept frozen, they could be transported even to distant places without seriously impairing their adrenaline contents. A cheaper, though less satisfactory method, which is practicable under the present-day conditions, would be to pack the glands (which have been sprinkled over with toluene) in ice and work them up in 2-3 days' time. Storage in alcohol also seems to hold out good prospects.

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1. Dey, Krishnan, Srinivasan, *Curr. Sci.*, 1943, **12**, 151.